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David W. Christianson  
Department of Chemistry  
University of Pennsylvania  
chris@sas.upenn.edu

## CRYSTAL STRUCTURE OF HUMAN ARGINASE I AT 1.29 Å RESOLUTION AND EXPLORATION OF INHIBITION IN THE IMMUNE RESPONSE

L. Di Costanzo<sup>1</sup>, G. Sabio<sup>2</sup>, A. Mora<sup>2</sup>, P.C. Rodríguez<sup>3</sup>, A.C. Ochoa<sup>3</sup>, F. Centeno<sup>2</sup>, and D.W. Christianson<sup>1</sup>

<sup>1</sup>Roy and Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania;

<sup>2</sup>Department de Bioquímica y Biología Molecular, Facultad de Veterinaria, Universidad de

Extremadura, Spain; <sup>3</sup>Tumor Immunology Program, Stanley S. Scott Cancer Center and Department of Pediatrics, Louisiana State University

*Human arginase I, an enzyme critical to the urea cycle, is a potential drug target for diseases linked to compromised L-arginine homeostasis. Here, we report high-affinity binding of the boronic acid-based inhibitors "ABH" and "BEC" to human arginase I. The 1.29 Å resolution structure of the complex with ABH yields an unprecedented view of arginase I's binuclear manganese cluster and illuminates the structural basis for nanomolar enzyme-inhibitor affinity. This work serves as a foundation for studying the structural and chemical biology of arginase I in the immune response, and we demonstrate the inhibition of arginase activity by ABH in human and murine myeloid cells (blood cells).*

Arginase is a trimeric binuclear manganese metalloenzyme that catalyzes the hydrolysis of the amino acid L-arginine to form L-ornithine and urea. Two arginase isozymes have been identified in mammals: cytosolic arginase I, which is responsible for the nitrogen-elimination step of the urea cycle, and mitochondrial arginase II, which functions in L-arginine homeostasis. Significantly, arginase inhibitors can enhance NO-dependent biological processes such as smooth muscle relaxation, so arginase is a potential drug target for diseases characterized by insufficient NO flux in biological signaling pathways. Most recently, our laboratory has determined that arginase is a potential drug target for the treatment of male and female sexual arousal disorders. Arginase also plays a role in mediating the effects of nitric oxide in the immune response. Notably, macrophage arginase I and NO synthase are reciprocally

regulated at the level of transcription: NO synthase is induced by TH1 cytokines, and arginase I is induced by TH2 cytokines. As a modulator of NO-dependent macrophage cytotoxicity, arginase I is implicated in the suppression of the tumoricidal activity of macrophages and T cells. In a murine model of multiple sclerosis, arginase I expression is up-regulated approximately 300-fold. Administration of the arginase inhibitor ABH improves the symptoms of the disease.

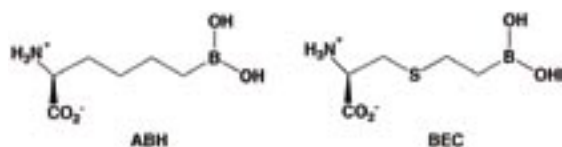
In the current study, we demonstrate that the boronic acid substrate analogues ABH and BEC (**Figure 1**) are highly potent inhibitors of human arginase I, with  $K_d$  values of 5 and 270 nM, respectively. Therefore, ABH and BEC may be potentially useful for blocking tumor-cell growth or treating multiple sclerosis in humans. Analysis of the high-resolution structures of human arginase I complexed with ABH and BEC reveals that the boronic acid moiety of each inhibitor undergoes a nucleophilic attack to bind as tetrahedral boronate anion (**Figure 2**). This binding mode mimics that expected for the actual tetrahedral intermediate and its flanking transition states in catalysis. Thus, boronic acid inhibitors such as ABH and BEC are reactive substrate analogues that are chemically transformed into transition state analogues in the enzyme active site. Such reactive substrate analogues are informally known as "reaction coordinate analogues."

Nanomolar affinity is a consequence of strong boronate-manganese coordination interactions as well as hydrogen-bond networks between the enzyme and the  $\alpha$ -carboxylate and  $\alpha$ -amino groups of the inhibitor, as illustrated for BEC in



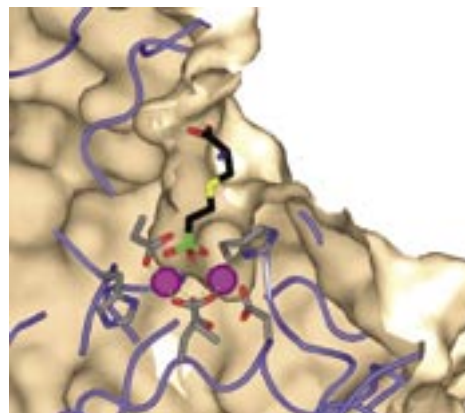
Authors Luigi Di Costanzo and David Christianson

**Figure 3.** In catalysis, this array of intermolecular interactions ensures specificity for L-arginine and disfavors binding and catalysis for L-arginine analogues bearing modified  $\alpha$ -substituents. These structure-affinity relationships are important to

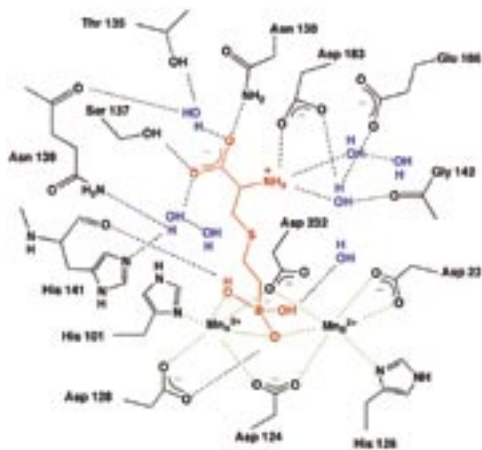


**Figure 1.** Human arginase inhibitors S-(2-boronethyl)-L-cysteine (BEC,  $K_d = 270$  nM) and 2(S)-amino-6-borohexanoic acid (ABH,  $K_d = 5.0$  nM).

consider as arginase I is explored as a potential target for new therapies directed toward inflammatory and immunological disorders, and especially so as boronic acid-based inhibitors ultimately join the growing family of enzyme-targeted drugs.



**Figure 2.** Complex between human arginase I and the reactive substrate analogue inhibitor S-(2-boronethyl)-L-cysteine (BEC). The inhibitor binds as the tetrahedral boronate anion (green). Coordination interactions with the binuclear manganese cluster (magenta) spheres are indicated by green dotted lines.



**Figure 3.** Summary of intermolecular interactions in the human arginase I-BEC complex.